

Table 1 - Study Variables: At each on-site visit, data of primary outcome (behaviour), secondary outcome (seizure semiology) as well as further data of the participants are collected (data gathered only at certain visits will be marked as: visit 0=V0, visit 1=V1, visit 2=V2). To evaluate the efficacy of the probiotic (*Bifidobacterium longum* BL999) in comparison to the placebo supplement the data will be categorised into preselected study variables and statistical analysis will be conducted as presented below. In addition, comparison groups are established from the following variables: study phase (phase 1 vs. Phase 2), study supplement (probiotic vs. placebo), responder rate (seizure frequency reduction of at least 50%) and seizure semiology (seizure type: occurrence of generalised and focal seizures; history/occurrence of cluster seizures, history/occurrence of status epilepticus). Adhered to previous studies based on a similar study design, the following table provides an overview of the planned statistical analysis [55, 56]. It is modified from Berk et al. 2019 [56].

Variable measuring points	Visit 0	1. Interim check-up	Visit 1	2. Interim check-up	Visit 2
Study day	0, before start of study	42±2	84±2	147±2	189±2

Study variable	Variable type		Variable type	Subtype	Statistical tests	
	Outcome	Covariate			Non-parametric	Parametric

Demographics						
Body weight (kg)	X		kg	continuous	Wilcoxon-signed rank test	Paired t-test
Age (years)		X	years	continuous	Wilcoxon-signed rank test	Paired t-test

Body temperature (°C)		X	°C	continuous	Wilcoxon-signed rank test	Paired t-test
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Examination						
Clinical	X	X	abnormal/normal	categorical	Spearman C	Chi-Square X ² test
Neurological: mentation, behaviour, body posture, gait, cranial nerves, postural reactions, spinal reflexes, sensory exam	X	X	abnormal/normal, grading (0=absent, 1=decreased, 2=normal, 3=increased, 4=clonus)	categorical	Spearman C	Chi-Square X ² test
Visual Analogue Scale (owner assessment of ataxia, sedation, sleep, overall quality of life)	X	X	0-100, change in %	continuous/ categorical	Wilcoxon-signed rank test; Spearman C	Paired t-test; Chi-Square X ² test

Seizure data						
Seizure frequency	X		N per months, study phase, change in %	continuous	Wilcoxon-signed rank test	Paired t-test
Seizure days	X		N per months, study phase, change in %	continuous	Wilcoxon-signed rank test	Paired t-test
Seizure types	X		presence/absence	categorical	Spearman C	Chi-Square X ² test

Cluster seizure days/occurrence	X		N per months, positive/negative, change in %	continuous/categorical	Wilcoxon-signed rank test; Spearman C; McNemar-test	Paired t-test; Chi-Square X ² test; McNemar-test
Status epilepticus	X		N per months, positive/negative	continuous/categorical	Wilcoxon-signed rank test; Spearman C; McNemar-test	Paired t-test; Chi-Square X ² test; McNemar-test
Inter-seizure interval	X		In days, months, change in %	continuous/categorical	Wilcoxon-signed rank test; Spearman C	Paired t-test; Chi-Square X ² test
Responder rate	X		% of seizure reduction, responder vs. non-responder	continuous/categorical	Wilcoxon-signed rank test; Spearman C	Paired t-test; Chi-Square X ² test

Blood analysis						
Haematology		X	relative, absolute values	continuous	Wilcoxon-signed rank test	Paired t-test
Blood chemistry		X	U/l, mmol/l, µmol/l, g/l	continuous	Wilcoxon-signed rank test	Paired t-test
Canine pancreatic lipase activity		X	µg/l	continuous	Wilcoxon-signed rank test	Paired t-test
Pre- and postprandial (only at V0) bile acid serum concentration	X		µmol/l	continuous	Wilcoxon-signed rank test	Paired t-test
Drug serum concentration		X	µg/ml	continuous	Wilcoxon-signed rank test	Paired t-test

Serotonin serum level	X		ng/ml	continuous	Wilcoxon-signed rank test	Paired t-test
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Other physiological variables						
Salvia cortisol levels	X		µg/dl	continuous	Wilcoxon-signed rank test	Paired t-test
Urinalysis (only at V0)	X		positive vs. negative; mg/dl; specific gravity	continuous/categorical	Wilcoxon-signed rank test; Spearman C	Paired t-test; Chi-Square X ² test
Neurotransmitter concentration in urine (only at V1 + V2): serotonin, histamine, glycine, phenylethylamine, dopamine, epinephrine, norepinephrine, glutamate, γ- aminobutyric acid	X		µmoles/g creatinine; µg/g creatinine; nmoles/g creatinine	continuous	Wilcoxon-signed rank test	Paired t-test
Metabolome	X		relative/absolute metabolite concentration; mmol/l; g/l	continuous	Wilcoxon-signed rank test	Paired t-test
Microbiome (only at V1 + V2)	X		Operational Taxonomic Units (OTUs); physiological/pathological; Dysbiosis Index (DI)	continuous/categorical	Wilcoxon-signed rank test; Spearman C	Paired t-test; Chi-Square X ² test

Questionnaire data						
ADHD RS [59]	X		4 items, option-scaled rating (1-4)	continuous/ categorical	N=2, Wilcoxon- signed rank test; N>2, Kruskal- Wallis-Test; Spearman C	N=2, Paired t-test; N>2, ANOVA; Chi- Square X ² test
DPQ [61]	X		5 items, option-scaled rating (1-5)	continuous/ categorical	N=2, Wilcoxon- signed rank test; N>2, Kruskal- Wallis-Test; Spearman C	N=2, Paired t-test; N>2, ANOVA; Chi- Square X ² test
CCDR [62]	X		score: 15-75, option-scaled rating (1-5)	continuous	N=2, Wilcoxon- signed rank test; N>2, Kruskal- Wallis-Test; Spearman C	N=2, Paired t-test; N>2, ANOVA; Chi- Square X ² test
C-BARQ [63]	X		11 groups, option-scaled rating (0-4)	continuous/ categorical	N=2, Wilcoxon- signed rank test; N>2, Kruskal- Wallis-Test; Spearman C	N=2, Paired t-test; N>2, ANOVA; Chi- Square X ² test
EpiQoL [64]	X		5 groups, option-scaled rating (1-5/0-5)	continuous/ categorical	N=2, Wilcoxon- signed rank test; N>2, Kruskal- Wallis-Test; Spearman C	N=2, Paired t-test; N>2, ANOVA; Chi- Square X ² test

Behavioural test						
Exploratory behaviour	X		distance, trait occurrence, frequency	continuous/categorical	Wilcoxon-signed rank test; Spearman C	Paired t-test; Chi-Square X ² test
Stranger-directed behaviour	X		distance, trait occurrence, frequency	continuous/categorical	Wilcoxon-signed rank test; Spearman C	Paired t-test; Chi-Square X ² test
Separation anxiety	X		distance, trait occurrence, frequency	continuous/categorical	Wilcoxon-signed rank test; Spearman C	Paired t-test; Chi-Square X ² test
Noise-related anxiety	X		distance, trait occurrence, frequency	continuous/categorical	Wilcoxon-signed rank test; Spearman C	Paired t-test; Chi-Square X ² test

List of abbreviations:

[°C: degree Celsius; N: value/amount; U: Unit; U/l: Unit per 1; %: per cent; ADHD RS: attention-deficit/hyperactivity disorder rating scale; DPQ: dog Personality Questionnaire; CCDR: canine cognitive dysfunction rating scale; C-BARQ: canine behavioural assessment & research questionnaire; EpiQoL: evaluation of quality of life in dogs with idiopathic epilepsy]

References

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